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Reassessment of central Peruvian Telmatobiinae (genera *Batrachophrynus* and *Telmatobius*). II. Allozymes and phylogenetic relationships

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Three hypotheses on the phylogenetic relationships among central Peruvian Telmatobiinae were tested: (1) the common ancestor of the two *Batrachophrynus* species diverged from the telmatobiine stock independently from the common ancestor of the present *Telmatobius*; (2) *B. macrostomus* and *B. brachydactylus* separated independently from the lineage leading to the present *Telmatobius*; (3) the separation of the common *Batrachophrynus* ancestor from *Telmatobius* occurred after the differentiation of the *Telmatobius* stock into geographical lineages. Allozymes clearly indicate the monophyly of *Batrachophrynus*, and that the southern Peruvian *T. culeus* is more closely related to the central Peruvian *T. jelskii* and *T. rimac* than to either of the *Batrachophrynus* species. All available evidence supports the first hypothesis.

INTRODUCTION

Three genera of central Peruvian Telmatobiinae are currently recognized: *Telmatobius* Wiegmann, 1835, *Batrachophrynus* Peters, 1873 and *Lynchophrys* Laurent, 1983 (FROST, 1985; DUELLMAN, 1993). A taxonomic reassessment of the six species assigned to these genera indicated that the monotypic genus *Lynchophrys* is not valid and that *Lynchophrys brachydactyla* should be referred to as *Batrachophrynus brachydactylus* (SINSCH et al., 1995). In contrast, osteological evidence (LYNCH, 1978) and morphometric data (SINSCH et al., 1995) continue to support the validity of the genera *Batrachophrynus* and *Telmatobius*. The corresponding phylogenetic hypothesis assumes a monophyletic origin of both genera (CEI, 1986).

LAURENT (1983), however, proposed an alternative hypothesis. He assumed an early separation of *B. macrostomus*, and an independent, but later, separation of *B. brachydactylus* from the *Telmatobius* stock. If this was true, the morphometric and osteological similarities between the two *Batrachophrynus* species would be convergences and the genus would be paraphyletic. There is a third possibility, considering the conspicuous general similarity between the two genera: the separation of the *Batrachophrynus* ancestor from the

central Peruvian *Telmatobius* stock may have occurred after the splitting of *Telmatobius* into a northern and a southern lineage. In this case, the genus *Batrachophrynus* would be invalid and its two species would have to be included in the genus *Telmatobius*.

The aim of our study was to test these alternative hypotheses by analyzing the genetic similarity among four central Peruvian and a southern Peruvian species of Telmatobiinae. The stream-dwelling *T. jelskii* (Peters, 1873), *T. rimac* Schmidt, 1954 and *B. brachydactylus* Peters, 1873, and the lake-dwelling *B. macrostomus* Peters, 1873, inhabit neighbouring regions within the Departments of Cerro de Pasco, Junín and Lima. The Titicaca frog *T. culeus* (Garman, 1875), which represents another evolutionary lineage within the genus *Telmatobius* (VELLARD, 1951, 1953, 1955), was included as a potential outgroup. Horizontal starch gel electrophoresis of the proteins of blood, muscle and liver homogenates was used to assess allelic variation in homologous loci among all populations and species. Our reconstruction of phylogenetic relationships within this Andean group of Telmatobiinae clearly supports CEI's (1986) hypothesis of a monophyletic origin of *Batrachophrynus* and *Telmatobius*.

MATERIAL AND METHODS

We examined 111 frogs representing five species of Telmatobiinae (Leptodactylidae): (1) *Batrachophrynus brachydactylus*: 11 males and 11 females from a brook near Ondores (Dep. Junín, Perú); (2) *B. macrostomus*: 6 males, 7 females and 8 juveniles from Junín Lake (Dep. Junín); (3) *Telmatobius jelskii*: (a) 5 males, 2 females and 14 juveniles from Rio Shullcas, Palian near Huancayo (Dep. Junín), (b) 6 males and 3 females from Cuyrohuasi near Tarma (Dep. Junín); (4) *T. rimac*: (a) 7 males, 7 females and 8 juveniles from Rio Chillón, Obrojillo near Canta (Dep. Lima, Perú), (b) 9 males, 4 females and 2 juveniles from Quebrada Huaytara, Canta (Dep. Lima); (5) *T. culeus*: 1 male from Titicaca Lake (Dep. Puno, Perú).

Following the morphological classification of specimens (SINSCH, 1986, 1990; SINSCH et al., 1995), we collected blood samples (about 60 µl per individual) from the vena angularis of the living frogs (NÖLLER, 1959). Samples were centrifuged at 11,500 rpm for 3 33 min and the cell fraction was dissolved in 20 µl homogenate buffer (tris-EDTA-NADP at pH 7.0) and stored at -18°C until use. The frogs were sacrificed and liver and muscle samples were taken and mechanically homogenated in 50-150 µl in tris-EDTA-NADP buffer at pH 7.0. All samples were collected in Perú in 1992 except for the blood sample of the *T. culeus* specimen. This individual was kept in the Museum of Natural History in Bonn, Germany, and was included to look for fixed alleles in the *Telmatobius* and *Batrachophrynus* lineages.

Gels for horizontal electrophoresis were prepared at 12 % with SIGMA starch. Four buffer systems were used at constant 55 mA and 4°C: (1) tris-citrate (electrode: pH 8.0; gel: pH 8.7), duration of electrophoresis: 5 h; (2) tris-malate (electrode and gel: pH 8.4), 4 h; (3) tris (electrode and gel: pH 8.6), 5 h; (4) tris-borate (electrode and gel: pH 9), 4 h. Each

gel was sliced into five 2 mm thick slabs for staining. Procedures for staining were those described by PASTEUR et al. (1988), SHAW & PRASAD (1970) and SHERIF (1990).

Allozymes examined were representative of 12 enzyme systems controlled by a total of 22 presumptive loci: aspartateamino transferase (1 locus, abbreviation: AAT, E.C. No. 2.6.1.1); adenylate kinase (1, AK, 2.7.4.3); esterase (4, EST, 3.1.1.1); glucosephosphate isomerase (2, GPI, 5.3.1.9); hexanol dehydrogenase (2, HDH, 1.1.1.56); isocitrate dehydrogenase (2, IDH, 1.1.1.42); lactate dehydrogenase (2, LDH, 1.1.1.27); malate dehydrogenase (2, MDH, 1.1.1.37); malic enzyme (1, ME, 1.1.1.40); peptidase (3, PEP, 3.4.1.1); 6-phosphogluconate dehydrogenase (1, 6-PGD, 1.1.1.44); phosphoglucomutase (2, PGM, 2.7.5.1). In addition, we scored the non-enzymatic hemoglobin (Hb).

Multiple loci were numbered from cathode to anode. Presumptive alleles were designated numerically according to their mobility relative to the most common electromorph (assigned 100) of the *T. jelskii* population from Palian. Faster moving electromorphs were assigned higher values (above 100), slower moving ones lower values (below 100). For reference, each electrophoretic run included samples of the *T. jelskii* population from Palian. Statistical analyses included the calculation of allele frequencies, of Nei's genetic distance (NEI, 1972), Cavalli-Sforza's chord distance (CAVALLI-SFORZA & EDWARDS, 1967) and Reynolds's genetic distance (REYNOLDS et al., 1983) by the program GENDIST 3.4 (package PHYLIP; FELSENSTEIN, 1985). Average heterozygosity per locus (Ho = observed frequency; He – expected frequency), proportion of polymorphic loci (P %), and the mean number of alleles per locus (A) were calculated for each sample except *T. culeus*. We used the G-test to detect deviations of observed heterozygosity from the Hardy-Weinberg equilibrium.

Reconstruction of phylogenetic relationships between the examined populations is based on four algorithms applied to allele frequencies: (1) UPGMA method (program NEIGHBOR 3.41); (2) Fitch-Margoliash method assuming equal rates of evolutionary change in all lineages (KITSCH 3.41); (3) Fitch-Margoliash method without evolutionary clock (FITCH 3.41); (4) maximum likelihood method (CONTML 4.42). All calculations are based on the cited programs in the package PHYLIP (FELSENSTEIN, 1985).

RESULTS

ALLELIC VARIATIONS OF PROTEINS

A total of 23 presumptive loci (enzymes: AAT, AK, EST, GPI, HDH, IDH, LDH, MDH, ME, PEP, 6PGD, PGM; non-enzymatic protein: Hb) was scored in 6 populations of four central Peruvian telmatobiine species and in one specimen of *T. culeus* (Table I). Five loci were monomorphic in all samples: HDH1, LDH2, PEP2.1, 6PGD and PGM1. Allele frequencies are listed in Table I. The observed heterozygosity significantly deviated from the expected heterozygosity of the Hardy-Weinberg equilibrium in all populations because of a deficit of heterozygotes at all loci (G-test, $P < 0.001$). The following account of the loci demonstrates that fixed alleles at the LDH1 locus permit an unequivocal

distinction between the genera *Batrachophrynus* and *Telmatobius*, and that the southern Peruvian *T. culeus* differs from all central Peruvian populations by the presence of an unusual allele at the MDH1 locus.

Aspartateamino transaminase

Following electrophoresis in the tris-malate system, we regularly detected activity in muscle and liver samples. The *Batrachophrynus* species are monomorphic for allele 100, whereas the *Telmatobius* populations possess a second, more slowly migrating AAT (allele 60). Heterozygotes were not observed.

Adenylate kinase

All blood and liver samples show stainable activity following electrophoresis in the tris-borate system. The frequencies of the main alleles 100 and 120 are similar in all samples. One specimen of *B. brachydactylus* possessed a third allele 75. Heterozygotes were not observed.

Esterase

We detected four loci which can easily be distinguished by their specific activity in different tissues and their electrophoretic mobility in the tris system. The most slowly moving esterase (EST1) usually shows low activity in the liver samples and sometimes also in the blood samples, whereas EST2 is active in all samples but stains most strongly in the liver samples. The faster moving esterases (EST3, EST4) produce stainable bands with about the same activity in all samples. The frequencies of the main alleles 90 and 100 of the EST1 locus are similar in all populations. Two *B. brachydactylus* showed a third allele. Two alleles 90 and 100 of EST2 locus are present in both *Batrachophrynus* species and in *T. rimac*, whereas in *T. jelskii* this locus is monomorphic. With the exception of two heterozygotes of the constitution 50/100 (*B. brachydactylus*) at the EST1 locus, all other specimens are homozygotes for the four esterase loci.

Glucosephosphate isomerase

In the tris-borate system an anodally migrating GPI was active in blood and liver samples. Allele 100 dominates in all populations, except for those of *T. rimac* which are almost monomorphic for allele 73. No heterozygotes were detected.

Hexanol dehydrogenase

The tris-malate system resolved two systems of HDH, the monomorphic HDH1 locus being active at similar levels in all tissues and the polymorphic HDH2 locus with stainable

activity in muscle and liver samples. The frequency of allele 100 is greater in the *Batrachophrynus* species than in the *Telmatobius* species where allele 75 dominates. A single heterozygote of the constitution 75/100 (*B. macrostomus*) was detected.

Isocitrate dehydrogenase

Two polymorphic loci coding for enzymes of considerably different electrophoretic mobility were detected in the tris-citrate system. Both loci were almost exclusively active in muscle and liver samples. In *Telmatobius* the IDH1 locus is monomorphic for the allele 100, whereas in *Batrachophrynus* a second, rare allele 93 is present. Four alleles are found at the IDH2 locus with allele 100 dominating in *Telmatobius*. In *Batrachophrynus* allele 90 is the most common one. A single heterozygote of the constitution 100/125 (*B. brachydactylus*) was detected.

Lactate dehydrogenase

Following electrophoresis in the tris-citrate system, only one band stained in the muscle and liver samples of all species, whereas in the blood samples up to five bands appeared. The common stainable band of all tissues is the tetramer of the unit coded for by the LDH1 locus. The five banded pattern was detected only in the blood of the two *Batrachophrynus* species, whereas in the blood of the *Telmatobius* species at most three bands stained, corresponding to slowly moving tetramers. The LDH1 locus is diagnostic for the distinction of the two genera: all *Telmatobius* are fixed for the allele 100, all *Batrachophrynus* for the allele 33. Pure tetramers of the unit coded for by the LDH2 locus were found in both *Batrachophrynus* with the same electrophoretic mobility (allele 100). The poor resolution of the one or two bands of mixed tetramers in *Telmatobius* does not permit a reliable estimate of the position of the non-expressed pure LDH2-tetramer.

Malate dehydrogenase

The best resolution of the bands corresponding to two polymorphic MDH loci was found in the tris-borate system. The MDH1 locus produces a slowly moving protein which was exclusively active in blood and liver samples. In contrast, the faster moving product of the MDH2 locus was present in all tissues, but, especially in the muscle samples, subbands frequently appeared which were not present in the other tissues of the same individual. The MDH1 locus is diagnostic for the southern Andean *T. culeus* which possesses allele 125, whereas only alleles 71 and 100 are present in all central Peruvian populations. The frequencies of allele 71 are extremely low in both *Batrachophrynus*, but considerably greater in *Telmatobius*. Three alleles are found at the MDH2 locus, the usually dominating allele 100, the less frequent allele 85 and the rare allele 115. Only one heterozygote of the constitution 85/100 (*T. rimac*, Obrojillo) was detected at the MDH2 locus.

Malic enzyme

The polymorphic ME locus accounted for regular activity in all samples following electrophoresis in the tris-malate system. Allele 100 dominates in all populations and reaches almost monomorphic frequencies in *T. jelskii*. *T. rimac* and the two *Batrachophrynus* differ from this species by a considerably higher frequency of allele 60 and the presence of the rare allele 20. At this locus we detected 12 heterozygotes: six of the constitution 20/60 (3 *B. brachydactylus*, 1 *B. macrostomus*, 2 *T. rimac* from Obrojillo), and another six of the constitution 60/100 (1 *B brachydactylus*, 1 *B. macrostomus*, 3 *T. rimac* from Obrojillo, 1 *T. rimac* from Huaytara).

Peptidase

Following electrophoresis in the tris-citrate system, we identified three PEP loci which were monomorphic in most populations. The peptidase of the PEP1 locus specifically digested the dipeptide VAL-LEU and had greater activity in blood and liver samples than in muscle tissue. The other two peptidases used the tripeptide LEU-GLY-GLY as a substrate, but the activity of the PEP2.1 locus was restricted to muscle and liver samples of *B. brachydactylus* and *T. rimac* and one specimen of *T. jelskii*, whereas the PEP2.2 locus was exclusively active in the blood samples of all populations (except *T. culeus*). The PEP1 locus is monomorphic in all but one species: *B. macrostomus* possesses a second allele 83 in low frequency. The PEP2.2 locus is monomorphic in all *Telmatobius* populations, but in the two *Batrachophrynus* species a second allele 115 occurs in low frequency. No heterozygotes were found at any of the loci.

6-phosphogluconate dehydrogenase

Following electrophoresis in the tris-citrate system, in all tissues we found activity corresponding to the same allele of a monomorphic locus.

Phosphoglucomutase

Enzyme systems corresponding to two loci were resolved in the tris-malate system. The monomorphic PGM1 locus stained with similar activity in all tissues, whereas the polymorphic PGM2 locus was detectable exclusively in the muscle and liver samples. In *Telmatobius* and *B. brachydactylus* allele 80 dominates, in *B. macrostomus* allele 100. A total of 6 heterozygotes was found: three of the constitution 80/100 (*T. rimac* from Obrojillo), one of 80/113 and two of 100/113 (*T. jelskii* from Palian).

Hemoglobin

In the tris system the distinction of bands corresponding to hemoglobin was best. We found two alleles present in all central Peruvian populations, allele 100 dominating in the *Telmatobius*, allele 120 in the *Batrachophrynus*. Heterozygotes were not detected.

Table I. - Allele frequencies at the polymorphic loci in 7 samples of 5 species of Andean Telmatobiinae (genera *Batrachophrynus* and *Telmatobius*). P %: relative frequency of polymorphic loci; A: average number of alleles per locus; H_e: relative frequency of expected heterozygosity; H_o: relative frequency of observed heterozygosity.

Locus + Alleles		*B. brachydactylus* N = 22	*B. macrostomus* N = 21	*T. jelskii* (Cuyro.) N = 9	*T. jelskii* (Palian) N = 21	*T. rimac* (Huaytara) N = 15	*T. rimac* (Obrojillo) N = 22	*T. culeus* N = 1
AAT	60	-	-	0.143	0 059	0 938	0.952	no activity detectable
	100	1 000	1.000	0.857	0.941	0.062	0 048	
AK	75	0 052	-	-	-	-	-	no activity detectable
	100	0 789	0.900	0.571	0.824	0.813	0.833	
	120	0 159	0.100	0 429	0.176	0.187	0.167	
EST1	50	0.077	-	-	-	-	-	-
	90	0 154	0.231	-	0 286	0 083	0 125	-
	100	0 769	0.769	1.000	0.714	0 917	0 875	1 000
EST2	90	0 105	0.053	-	-	0 125	0 111	-
	100	0 895	0.947	1.000	1 000	0 875	0 889	1 000
EST3	94	0.111	0.421	0.429	0 063	0 125	0 167	-
	100	0.889	0.579	0.571	0 937	0 875	0 833	1 000
EST4	92	-	0.053	-	0.067	0.125	0.412	-
	100	1 000	0.947	1.000	0 933	0 875	0.589	1 000
GPI	73	0 091	0 050	0.333	0 278	1.000	0.950	-
	100	0 909	0 950	0 667	0.722	-	0.050	1.000
HDH	75	0.545	0.395	0.750	0 850	0 875	0.579	no activity detectable
	100	0.455	0.605	0.250	0.150	0.125	0.421	
IDH	93	0 091	0.050	-	-	-	-	no activity detectable
	100	0 909	0.950	1.000	1.000	1.000	1.000	
IDH	90	0.600	0.400	-	-	-	0.071	no activity detectable
	100	0.300	-	1.000	0 667	1.000	0.929	
	110	-	0 400	-	-	-	-	
	125	0.100	0.200	-	0.333	-	-	
LDH1	33	1.000	1 000	-	-	-	-	-
	100	-	-	1 000	1 000	1.000	1 000	1.000
MDH1	71	0.050	0 067	0 444	0 188	0.688	0.556	-
	100	0.950	0 933	0.556	0 912	0.312	0.444	-
	125	-	-	-	-	-	-	1 000
MDH2	85	0.111	0 167	0 500	0.400	0.563	0.262	-
	100	0.833	0 750	-	0.600	0.437	0 643	1.000
	115	0 056	0 083	0 500	-	-	0 095	-
ME	20	0 167	0.026	-	-	-	0.053	-
	60	0.286	0.474	-	0.063	0.438	0 395	-
	100	0.547	0 500	1.000	0.937	0.562	0 552	1.000
PEP1	83	-	0 125	-	-	-	-	-
	100	1 000	0.875	1.000	1.000	1.000	1.000	1.000
PEP2 2	100	0 714	0.750	1.000	1.000	1.000	1.000	no activity detectable
	115	0 286	0.250	-	-	-	-	
PGM2	80	0.611	0.400	0.857	0 500	1.000	0 750	no activity detectable
	100	0.389	0.600	-	0.333	-	0.250	
	113	-	-	0.143	0 167	-	-	
Hb	100	0.136	0.444	0 889	0 455	0 875	0.563	1.000
	120	0 864	0.556	0.111	0 545	0.125	0.437	-
P %		0.61	0 68	0 41	0 62	0 50	0.64	
A		1.80	1 86	1 41	1 67	1.50	1.73	
H_e		0.30	0 29	0.39	0.30	0 28	0.31	
H_o		0.04	0 01	0 00	0.03	0.01	0.04	

Table II - Matrix of genetic distances among six samples of four central Peruvian species of Andean Telmatobiinae (genera *Batrachophrynus* and *Telmatobius*).

A. Nei's genetic distance

Species + Population	*B. macrostomus*	*T. jelskii* (Cuyro)	*T. jelskii* (Palian)	*T. rimac* (Huaytara)	*T. rimac* (Obrojillo)
B. brachydactylus	0.0301	0.2237	0.1204	0 3177	0.2613
B. macrostomus	-	0 2304	0.1417	0.3464	0.2874
T. jelskii (Cuyro)		-	0.0661	0 1055	0.1296
T. jelskii (Palian)			-	0.1387	0 1232
T rimac (Huaytara)				-	0 0245

B. Cavalli-Sforza's chord distance

Species + Population	*B. macrostomus*	*T. jelskii* (Cuyro)	*T. jelskii* (Palian)	*T rimac* (Huaytara)	*T. rimac* (Obrojillo)
B. brachydactylus	0.1211	0.5993	0.3667	0.6996	0.5434
B. macrostomus	-	0 6621	0.4227	0.7768	0 6131
T. jelskii (Cuyro.)		-	0.2349	0.3092	0.3408
T jelskii (Palian)			-	0.3123	0.2641
T rimac (Huaytara)				-	0.803

C. Reynolds's genetic distance

Species + Population	*B. macrostomus*	*T. jelskii* (Cuyro.)	*T. jelskii* (Palian)	*T rimac* (Huaytara)	*T. rimac* (Obrojillo)
B. brachydactylus	0 0895	0 4575	0.3009	0.5494	0.4481
B. macrostomus	-	0 4443	0.3174	0.5458	0 4501
T. jelskii (Cuyro)		-	0 2238	0.3512	0.3365
T. jelskii (Palian)			-	0.3840	0 3035
T rimac (Huaytara)				-	0 1004

Table III - Matrix of Nei's genetic distances among four north Peruvian *Telmatobius* species, calculated from the data published in WIENS (1993).

Species	*Telmatobius latirostris*	*Telmatobius necopinus*	*Telmatobius truebae*
Telmatobius brevipes	0.9246	0 8504	0 8702
Telmatobius latirostris	-	0.4479	0 3411
Telmatobius necopinus		-	0.4973

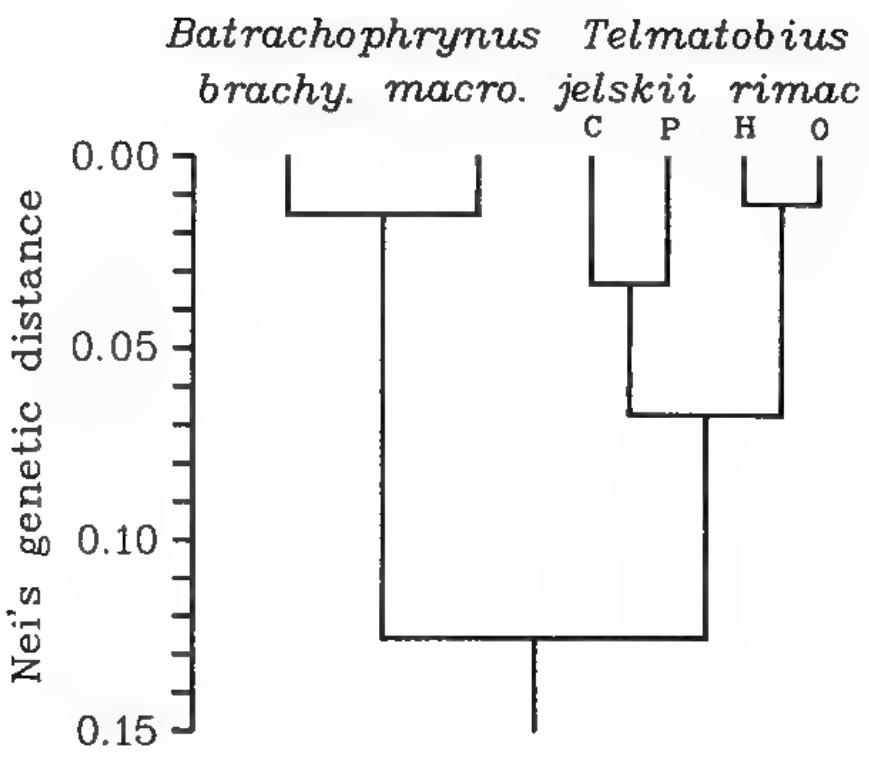

Fig. 1. – UPGMA dendrogram of genetic similarity among six samples of four central Peruvian species of Andean Telmatobiinae, based on the matrix of Nei's genetic distances (Table II A).

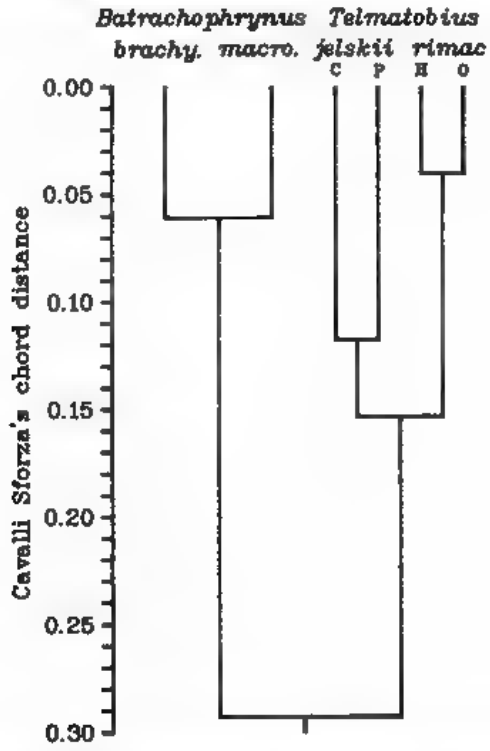

Fig. 2. – UPGMA dendrogram of genetic similarity among six samples of four central Peruvian species of Andean Telmatobiinae, based on the matrix of Cavalli-Sforza's chord distances (Table II B).

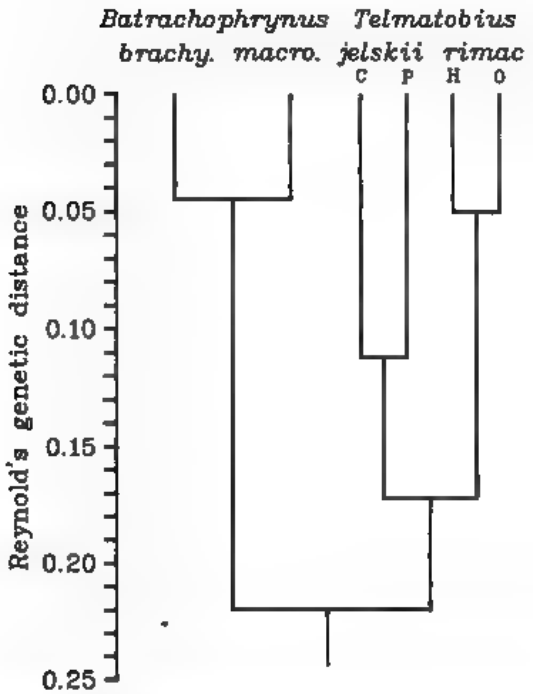

Fig. 3. – UPGMA dendrogram of genetic similarity among six samples of four central Peruvian species of Andean Telmatobiinae, based on the matrix of Reynolds's genetic distances (Table II C).

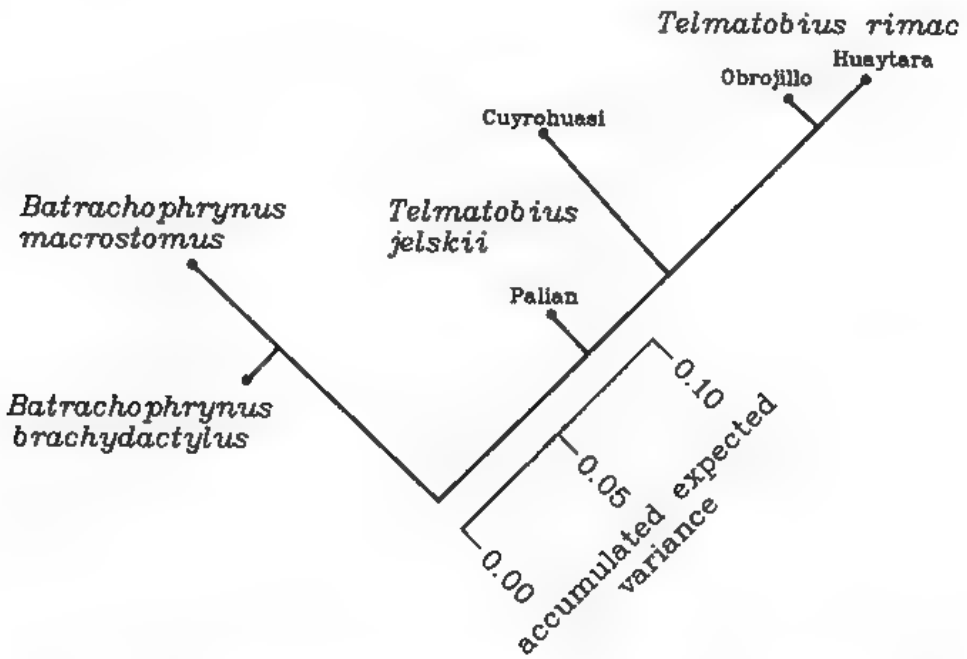

Fig. 4. – Genetic relationships among six samples of four central Peruvian species of Andean Telmatobiinae. Due to the absence of an outgroup the tree is arbitrarily rooted at the mean distance between the most similar populations of *Batrachophrynus* and *Telmatobius*. Maximum likelihood method, based on the allele frequencies (Table I); best tree out of 202 examined in five runs: ln likelihood = 88 3.

Phylogenetic relationships

Allele frequencies (Table I) obtained for the six populations of four central Peruvian telmatobiine species were used for the calculation of three measures of genetic distances (Table II). The reconstruction of the phylogenetic relationships by four commonly used algorithms was based on either allele frequencies or distance matrices (figs. 1-4).

Independent of the algorithm used for the calculation of the genetic distances among the populations sampled, the matrices obtained shared the following features: (1) the genetic differentiation between the two populations of *T. rimac* and between the two *Batrachophrynus* species was low and at the same level; (2) the level of differentiation between the two *T. jelskii* populations was 2-3 times greater than between the *T. rimac* populations, and the *Batrachophrynus* species; (3) the *Telmatobius* species were genetically more similar to each other than any to the *Batrachophrynus* species.

All algorithms based on distance matrices produced identical groupings of populations. Therefore, only the UPGMA-dendrograms are shown as representative examples (figs. 1-3). The main clusters corresponded to the genera *Batrachophrynus* and *Telmatobius* and conspecific populations were placed together. The unrooted maximum likelihood tree based on allele frequencies shows a similar grouping of the populations (fig. 4). However, the *T. jelskii* populations are placed on different branches of the *Telmatobius* lineage.

In addition to the four central Peruvian species, we also included the southern Peruvian *T. culeus* into the analysis. As the data on the allozymes expressed in this species are based on one individual, a quantitative approach to the phylogenetic relationships is not possible yet. Nevertheless, a qualitative reconstruction based on the presence or absence of alleles was attempted (fig. 5). Four monomorphic loci and several common alleles at the polymorphic loci justify the common root of the dendrogram. The distinction between *Batrachophrynus* and *Telmatobius* is based on fixed alleles at the LDH1 locus. *T. culeus* differs from *T. jelskii* and *T. rimac* by fixed alleles at the MDH1 locus. In *B. brachydactylus* and *B. macrostomus*, and in *T. jelskii* and *T. rimac*, respectively, we were unable to find a locus with different fixed alleles. Species distinction is based on the presence and absence, respectively, of rare alleles at the EST2-locus and the PEP1-locus.

Discussion

The radiation of a late tertiary stock of telmatobiine frogs into the recently uplifted cordilleran environments led to a differentiation of about 30 presently known species (Cei, 1986; Duellman, 1993; Frost, 1985; Wiens, 1993). Allopatric populations which inhabit numerous interandean valleys and streams of the Pacific or Atlantic hydrographic systems give an idea of the mechanisms of speciation at work. The geomorphologically complicated Late Pleistocene landscape favoured the formation of disjunct populations (Cei, 1986). Progressive genetic changes in isolated gene pools probably promoted allopatric speciation in the Andean Telmatobiinae. The tendency for homozygosity at almost all loci in the Andean populations already studied is a strong indication of small

population size and interrupted gene flow between populations. Large genetic distances between populations assigned to *T. jelskii* and inhabiting different river systems demonstrate that allopatric speciation is still the norm at the present time.

Early attempts to analyze relationships between the Andean Telmatobiinae (VELLARD, 1951, 1953, 1955) mainly reflect groupings assigned by biogeographical convenience to a poor data base. LYNCH (1978) was the first to use a cladistic approach on a set of morphological and osteological data. He placed the Andean genera *Batrachophrynus* and *Telmatobius*, along with additional seven genera (*Alsodes*, *Atelognathus*, *Eupsophus*, *Hylorina*, *Insuetophrynus*, *Limnomedusa* and *Somuncuria*), into the tribe Telmatobiini Fitzinger, 1843. All cladograms (based on differing numbers of OTUs) emphasized that the common ancestor of the lineage leading to the two *Batrachophrynus* species separated at a very early stage from the lineage leading to the present *Telmatobius* species. Nevertheless, DUELLMAN's (1979: 424) statement: "the systematic relationships of the species of *Telmatobius* presently are too poorly known to assess fully the historical biogeography of the group" remains valid in spite of increased knowledge on the Andean Telmatobiinae accumulated since VELLARD's pioneer work.

Our attempt to reconstruct phylogenetic relationships of the central Peruvian Telmatobiinae is based on allozymes. The results correspond to those on northern Peruvian *Telmatobius* (21 individuals, 4 species, 19 loci) in terms of low heterozygosity at most loci (WIENS, 1993). However, the Nei's distances among the species which we studied are generally lower than those among four northern Peruvian *Telmatobius* species (Table III, calculated from Table 5 in WIENS, 1993). This discrepancy is probably due to differences in method (e. g., number and kind of loci scored, number of individuals, buffer systems, resolution of gels). All dendrograms and trees derived from processing allele frequencies and distance matrices indicate the same sequence of speciation events among the four species of *Batrachophrynus* and *Telmatobius* we examined.

Allozymes clearly support monophyly in *Batrachophrynus*. The gene pools of *B. brachydactylus* and *B. macrostomus* were surprisingly similar and the genetic distance of 0.03 between the two species is the lowest interspecific Nei's distance ever reported for Amphibia (usually 0.1 to 3.0; AVISE & AQUADRO, 1982). LAURENT's (1983) hypothesis of an independent derivation from the *Telmatobius* stock is not supported. This hypothesis apparently resulted from a misinterpretation of convergent morphological traits in the stream-inhabiting *B. brachydactylus* and *Telmatobius* species.

We were unable to detect any fixed genetic difference between the two *Batrachophrynus* species and even allele frequencies are very similar. Taken alone, allozymes would suggest that *B. brachydactylus* and *B. macrostomus* are conspecific. On the other hand, these taxa are morphologically well defined, extremely different in size, and they live in different habitats (PETERS, 1873; SINSCH, 1990; SINSCH et al., 1995). Intermediate individuals between the *brachydactylus* phenotype and the *macrostomus* phenotype are not known. Personal field observations in the area of Lake Junín, where both taxa occur sympatrically, did not yield any evidence of interbreeding. In conclusion, despite the low differentiation of the *Batrachophrynus* gene pool for the allozymes we studied (comparable to studies on bird allozymes), we do not doubt the specific status of both taxa which we consider sister species.

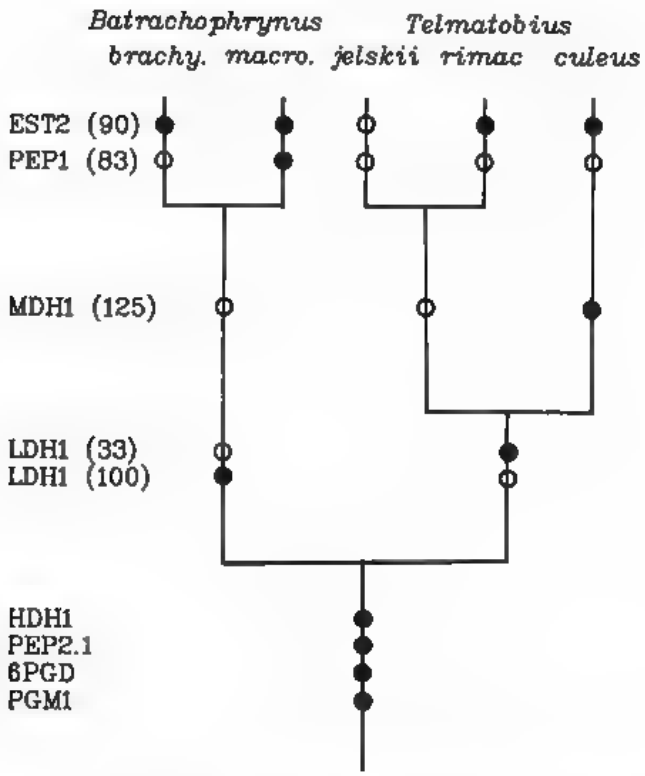

Fig. 5. – Proposal for the phylogenetic relationships among five species of Andean Telmatobiinae, based on the presence (dot) and absence (circle), respectively, of alleles at different allozyme loci.

The cluster formed by *Telmatobius* samples agrees with the morphometric assignment of populations to the species *T. jelskii* and *T. rimac*. The genetic distance between the conspecific samples correlates with the corresponding geographical distance of localities. Finally, the low genetic distance between *T. jelskii* and *T. rimac* as well as the absence of diagnostic fixed alleles indicates a close phylogenetic relationship.

The evaluation of fixed alleles and of presence/absence of rare alleles supports the same tree structure as the quantitative treatment of allele frequencies. Moreover, it allows for a proposal on the relationships of *T. culeus* with the central Peruvian taxa (fig. 5). Yet, the large geographical distance between the current distribution ranges of *T. jelskii* and *T. rimac* on one hand, and *T. culeus* on the other, indicates a long period of independent evolution. Nevertheless, the fixed allele at the LDH1 locus clearly suggests that *T. culeus* is a member of the same *Telmatobius* lineage as are the central Peruvian species. This shared character state distinguishes *Telmatobius* from *Batrachophrynus* and favours the hypothesis of an early separation put forward by LYNCH (1978). The geographically distant *T. culeus* genetically and morphometrically resembles the *Batrachophrynus* species more than the neighboring central Peruvian *Telmatobius* species do. This may indicate that *Batrachophrynus* species represent the remnants of an early invasion into the central Peruvian Andes, whereas the *Telmatobius* species reached this region during a second, later invasion. The limited present range of distribution of *Batrachophrynus*, and the suspicious

absence of streams occupied by both *B. brachydactylus* and a stream-inhabiting *Telmatobius* species, suggest that *Telmatobius* is competitively superior to *Batrachophrynus*. Future field work in the contact zone between *B. brachydactylus* and *T. jelskii* in the streams of the Junín area should reveal whether the two can coexist in the same stream.

Our study supports the taxonomic distinction of the central Peruvian Telmatobiinae into two genera *Batrachophrynus* and *Telmatobius* (PETERS, 1873). The geographical distribution of *Batrachophrynus* and *Telmatobius* species indicates the result of competition between early and late invaders of this region rather than phylogenetic proximity. However, the genetic distance between the members of these genera is low. Data from additional *Telmatobius* species and from an appropriate outgroup (*Alsodes*, considered as sister taxon of *Telmatobius*) are needed for a final decision on the relationships between *Batrachophrynus* and *Telmatobius*.

RESUMEN

Se revisan tres hipótesis sobre el origen filogenético de los Telmatobiinae del Perú central: (1) el antepasado común de las dos especies de *Batrachophrynus* se separó del stock de *Telmatobius* antes de su diferenciación en las especies recientes; (2) *B. macrostomus* se separó primero, *B. brachydactylus* más tarde del stock de *Telmatobius*; (3) la separación del antepasado común de *Batrachophrynus* del stock de *Telmatobius* ocurrió despues de su diferenciación. Alocimas demuestran claramente que *B. brachydactylus* y *B. macrostomus* son familiares muy cercanos, y que *T. culeus* del sur del Perú es relacionado más cercano con *T. jelskii* y *T. rimac* del Perú central que con las especies de *Batrachophrynus*. En conclusión, todas las pruebas disponibles apoyan la primera hipótesis.

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